

ANTIBIOTIC SUSCEPTIBILITY PATTERN AND MULTIPLE ANTIBIOTIC RESISTANCE INDEX OF *PSEUDOMONAS AERUGINOSA* URINE ISOLATES FROM A UNIVERSITY TEACHING HOSPITAL

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Urine samples submitted to the Medical Microbiology diagnostic laboratory of the Ahmadu Bello University Teaching Hospital, Zaria, were routinely screened for *Pseudomonas aeruginosa* over a three-month period with 13/150 (8.67%) of the pathogenic bacteria isolated positively identified. All the isolates were resistant to the cheap, commonly available antibiotics; rifampicin, ampicillin/cloxacillin, erythromycin, chloramphenicol and ampicillin but were uniformly susceptible to ciprofloxacin. The high prevalence of multidrug resistance indicates a serious need for broad-based, local antimicrobial resistance surveillance for continuous tracking of antibiotic resistance trends among all clinically relevant isolates and introduction of effective interventions to reduce multidrug resistance in such pathogens.

Key words: *Pseudomonas aeruginosa*, antibiotic susceptibility, multiple antibiotic resistance, urinary tract infection

INTRODUCTION

Pseudomonas aeruginosa is an opportunistic pathogen, which is highly resistant to antibiotic therapy (1). Previously, *Pseudomonas aeruginosa* was rarely considered as a real pathogen (2), perhaps because despite abundant opportunities to spread, its ability to survive in almost any environment, its innate resistance to many antibiotics or disinfectants and its array of putative virulence factors, *Pseudomonas aeruginosa* rarely causes community acquired infections in immunocompetent individuals (3).

Nowadays, however, it is among the most common pathogens involved in nosocomial infections (4), and has been described as a lethal pathogen with 34% of bacteraemia mortality attributable to it, a crude mortality of 50% in the bacteraemic

neutropaenic host and an overall mortality of 45% and 69% respectively, in bacteraemic nosocomial pneumonia and pneumonia in mechanically ventilated patients (2).

Pseudomonas aeruginosa has also been isolated as a pathogen responsible for urinary tract infections (UTIs) representing 10.7% of isolates found exclusively in nosocomial UTIs (5), 3.5% in intensive care units, 35.6% in other hospital units and 27.7% in out-patients and general practice (6).

It has been noted that antimicrobial resistance is a global concern (7). There is need for accurate and up-to-date information regarding the frequency of resistance, resistance trends and comprehensive comparison of various antimicrobial agents tested against different pathogens. Moreover, the case with which resistance develops to traditionally used anti-pseudomonads (due to

mutation, acquisition of plasmids, possession of intrinsic resistance factors) has increased dramatically in recent years. The possession of efflux pump systems capable of conferring resistance to a wide range of unrelated classes of antimicrobial agents has also been demonstrated in *Pseudomonas aeruginosa* (1).

There is therefore the need for antimicrobial sensitivity testing to be done routinely and accurately as guide to clinical judgments in the chemotherapy of *Pseudomonas aeruginosa* infections. In this paper, we report the results of antibiotic susceptibility and multiple antibiotic resistance (MAR) index of *Pseudomonas aeruginosa* isolates obtained from urine samples in the Ahmadu Bello University Teaching Hospital, Zaria, Nigeria.

MATERIALS AND METHODS

Isolation and Characterization

Urine samples submitted to the Medical Microbiology diagnostic laboratory of the Ahmadu Bello University Teaching Hospital, Zaria were routinely screened for *Pseudomonas aeruginosa* over a three-month period. Samples inoculated into sterile peptone water were grown overnight at 37°C and subcultures made into appropriate selective and diagnostic media. All isolates which produced colonies that were non-lactose fermenting, colourless or with shades of greenish pigments on MacConkey or Nutrient agars, oxidase positive, and Gram-negative rods, were subcultured on Pseudosel® agar. Isolates which were able to grow on this agar producing greenish or brownish colonies with foul smelling odour, and failed to ferment any of glucose, lactose, arabinose, sucrose, mannitol and xylose; but possessed such biochemical characters as determined with

Kligler iron agar, ability to utilize urea, citrate as well as positive catalase test were identified as *Pseudomonas aeruginosa* (8,9).

Antimicrobial Susceptibility Testing

The antibiotic susceptibility pattern of the isolates was determined using the agar diffusion plate method as described by the National Committee for Clinical Laboratory Standards (10). The antibiotics used were; ampicillin 30 µg, rifampicin 10 µg; ampicillin/cloxacillin 30 µg, ciprofloxacin 10 µg, gentamicin 10 µg, streptomycin 30 µg, erythromycin 30 µg and chloramphenicol 20 µg. *Pseudomonas aeruginosa* NCTC 10662 served as control.

Determination of MAR index

The MAR index was determined for each isolate by dividing the number of antibiotics to which the isolate is resistant by the total number of antibiotics tested (11,12).

RESULTS

A total of 213 urine samples were screened within the study period with 150 pathogenic bacteria isolated. Thirteen of the 150 pathogenic bacteria isolated were identified as *Pseudomonas aeruginosa* giving a positive rate of 8.6%. All the *Pseudomonas aeruginosa* isolates were resistant to rifampicin, ampicillin / cloxacillin, erythromycin, chloramphenicol and ampicillin. However, 77%, 92.3% and 100% were susceptible to streptomycin, gentamicin and ciprofloxacin respectively (Fig.1). All the isolates were found to be multi-resistant with MAR index of at least 0.625 (Table 1). Three distinct resistant patterns were identified and presented in Table 2.

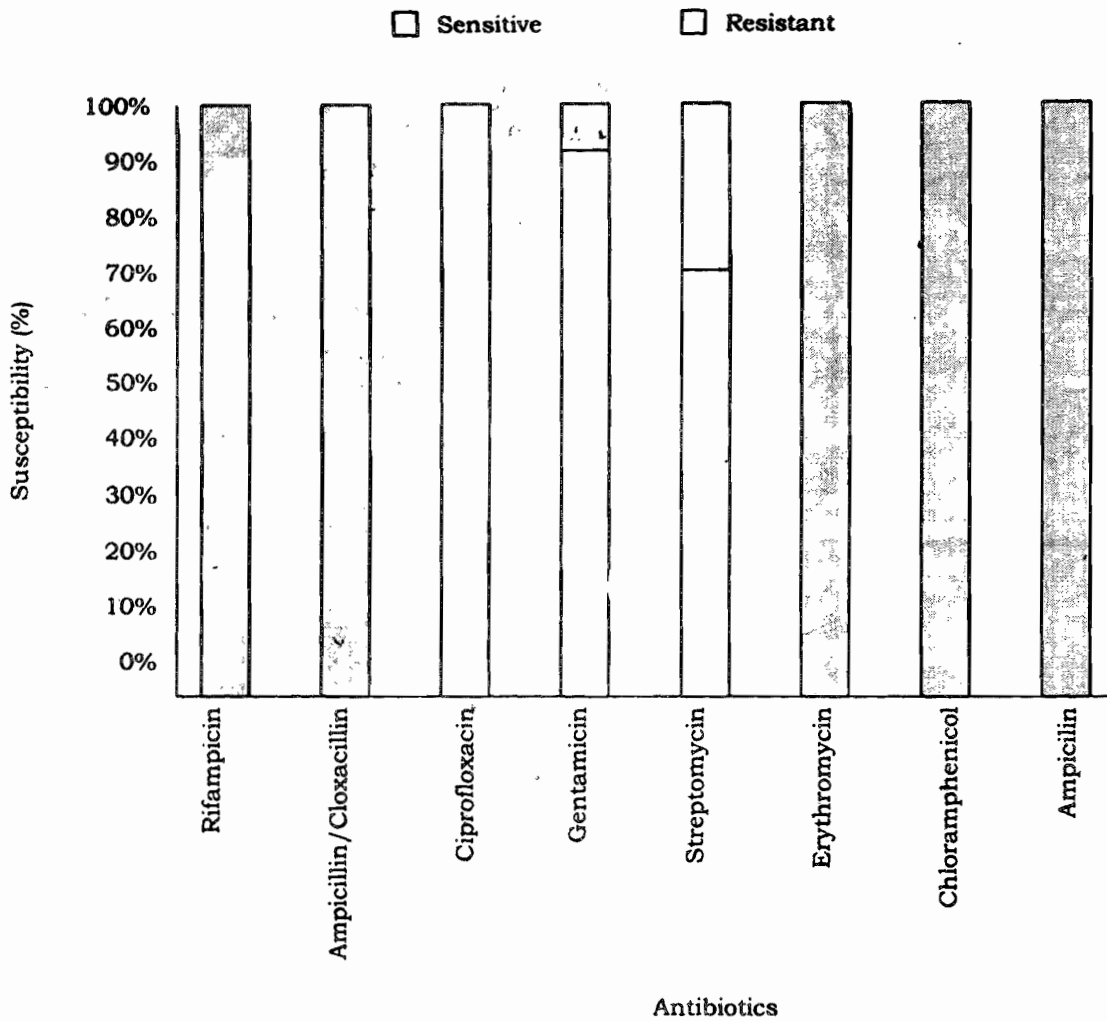


Fig. 1: Antibiotic susceptibility pattern of *Pseudomonas aeruginosa* isolates

Table 1: Multiple antibiotic resistance (MAR) index of *Pseudomonas aeruginosa* isolates

MAR Index	No of isolates	%
0.625	10	76.923
0.75	2	15.385
0.875	1	7.629
TOTAL	13	100

Table 2: Resistance patterns in *Pseudomonas aeruginosa* isolates

Resistance Pattern	%
Rif ^R , Apx ^R , Gent ^R , Strep ^R , Eryth ^R , Chl ^R , Amp ^R ,	7.692
Rif ^R , Apx ^R , Eryth ^R , Chl ^R , Amp ^R ,	76.923
Rif ^R , Apx ^R , Strep ^R , Eryth ^R , Chl ^R , Amp ^R ,	15.385
TOTAL	100

DISCUSSION

Previous studies in this environment have confirmed urine to be the leading source of *Pseudomonas aeruginosa* in hospital settings (13). This observation was attributed to the fact that most patients going for surgery tend to get catheterized, a procedure which has been shown to create inherent risks for infections (14). A prevalence rate 8.67% was observed for the isolation of *Pseudomonas aeruginosa* as a urinary pathogen in this study. This was higher than an earlier report of 6.4% prevalence in blood cultures (15), but agrees with 14.4% (all laboratory specimens) of Oduyebo, *et al* (16), and the 10.5% of Olayinka (13). The seeming fluctuation in prevalence rates in these studies was probably due to the differences in sample size and the durations of sampling.

About 7.7% of *Pseudomonas aeruginosa* isolates studied in this work were found to be resistant to gentamicin. This is significant because gentamicin is traditionally considered in this environment as the first line drug against Gram-negative bacilli in the hospital setting (16). The level of resistance in this study to streptomycin (another aminoglycoside) is 23%. There is little conclusive information on the mechanism of *Pseudomonas aeruginosa* resistance to aminoglycosides; however, the observed resistance to the penicillins (ampicillin 100%, ampicillin/cloxacillin 100%) in this study may not be unconnected with the well-known fact that *Pseudomonas aeruginosa* is intrinsically resistant to the penicillins and other antimicrobial agents (17).

The *Pseudomonas aeruginosa* isolates were uniformly susceptible to ciprofloxacin (a fluoroquinolone) underlying the need for the

rational use of antibiotics, as it is known that new and costly antibiotics are less available for abuse/misuse (16). The multiple antibiotic resistance (MAR) index gives an indirect suggestion of the probable source(s) of an organism. According to previous workers, MAR index greater than 0.2 indicates that an organism must have originated from an environment where antibiotics are often used (11,12), and as evident in Table 1, all of the isolates have MAR index far greater than 0.2 and are resistant to at least five antibiotics (Table 2).

Some of the mechanisms of antimicrobial resistance in *Pseudomonas aeruginosa* have been well studied (4,6). Some of the inherent resistance factors are impermeability, multi-drug efflux pump systems and a chromosomal *AmpC*-lactamase. Resistance to β -lactams and aminoglycosides can also arise from the acquisition of plasmids, transposons or integrons encoding β -lactamases and aminoglycoside-modifying enzymes (6). In addition to the constitutive low level of susceptibility of this organism to antimicrobial agents, new mechanisms of resistance have been identified, which include the production of β -lactamases (18). In the present study, 38.46% of *Pseudomonas aeruginosa* isolates produced β -lactamases. The significance of this observation derives from the knowledge that some of these enzymes can hydrolyze β -lactam agents (4).

At present, there are no strict rules concerning antibiotic prescriptions in this hospital, but in view of the fact that concerns are mounting about the spread of antimicrobial-resistant strains of microorganisms the world over, continued surveillance of antibiotic-resistance profile and

the effective communication of same to all involved in the use of antibiotics appears mandatory. Similarly, the high prevalence of multidrug-resistant strains of *Pseudomonas aeruginosa* in this study underscores the need for effective control measures in this environment.

REFERENCES

1. Lomovskaya O, Warren MS, Lee A, et al. Identification and characterization of inhibitors of Multidrug Resistance Efflux Pumps in *Pseudomonas aeruginosa*: Novel Agents for Combination Therapy. *Antimicrob. Agents. Chemother.* 2001; **45**(1): 105-116.
2. Giamarellou H. Prescribing guidelines for severe *Pseudomonas aeruginosa* infections. *J. Antimicrob. Chemother.* 2002; **49**: 229-233
3. Neu HC. The role of *Pseudomonas aeruginosa* in infection. *J. Antimicrob. Chemother.* 1983; **11**(Suppl B): 1-3
4. Pellegrino FLPC, Teixeira LM, Carvalho MGS, et al. Occurrence of Multidrug-Resistant *Pseudomonas aeruginosa* clone in different hospitals in Rio de Janeiro, Brazil. *J. Clin. Microbiol.* 2002; **40**(7): 2420-2424
5. Hryniewicz K, Szczypa K, Sulikowska A, Jankowski K, Betlejewska K, Hryniewica W. Antibiotic susceptibility of bacterial strains isolated from urinary tract infections in Poland. *J. Antimicrob. Chemother.* 2001; **45**: 773-780
6. Henwood CJ, Livermore DM, James D, Warner M and the Pseudomonas Study Group. Antimicrobial susceptibility of *Pseudomonas aeruginosa*: results of a UK survey and evaluation of the British Society of Antimicrobial Chemotherapy disc susceptibility test. *J. Antimicrob. Chemother.* 2001; **47**: 789-799
7. Blondeau JM, Yaschuk Y and the Canadian Multi-center Study Group. Canadian Multi-center Susceptibility Study, with a focus on Cephalosporins from Canadian Medical Centres. *Antimicrob. Agents. Chemother.* 1997; **41** (12): 2773-2775
8. Statford BC. An Atlas of Medical Microbiology: Common Human Pathogens. Blackwell Scientific Publications, Oxford, London, 1977.
9. Cheesbrough M. Medical Laboratory Manual for Tropical Countries. Vol. II Microbiology. Butterworth Heineman Ltd, Oxford, England, 1993.
10. Lennette EH, Balows P, Hausler WJ Jr, Shadomy HJ. Manual of Clinical Microbiology. 5th Edition, American Society of Microbiology, Washington DC, 1990
11. Krumperman PH. Multiple antibiotic indexing *Escherichia coli* to identifying risk sources of faecal contamination of foods. *Appl. Envir. Microbiol.* 1983; **46**: 165-170
12. Paul S, Bezbarauh RL, Roy MK, Ghosh AC. Multiple antibiotic resistance (MAR) index and its reversion in *Pseudomonas aeruginosa*. (Lett). *Appl Microbiol.* 1997; **24**: 169-171
13. Olayinka AT. The Prevalence, Antibiotic Susceptibility and Plasmid profiles of *Pseudomonas aeruginosa* in the Surgical Units of the Ahmadu Bello Teaching Hospital, Zaria. FMCPATH Dissertation. National Postgraduate Medical College of Nigeria, 2002.
14. Wong ES. Guidelines for the prevention of urinary tract infections. *Am. J. Infect. Contr.* 1983; **11**: 28-31
15. Adedare TA, Ibrahim YKE, Ehinmidu JO, Olayinka AT, Olayinka BO. Multiple Antibiotic Resistance (MAR) in *Pseudomonas* species isolated from blood of suspected typhoid patients. *Nig. J. Exper. Appl. Biol.* 2002; **3** (2) (In Press)
16. Oduyebo O, Ogunsoola FT, Odugbemi T. Prevalence of multi-resistant strains of *Pseudomonas aeruginosa* isolated at the Lagos University Teaching Hospital from 1994-1996. *Nig. Qt. J. Hosp. Med.* 1997; **7**: 373-376.
17. Okamoto K, Gotoh N, Nishino T. *Pseudomonas aeruginosa* reveals high intrinsic resistance to Penem antibiotics: penem resistance mechanisms and their interplay. *Antimicrob. Agents Chemother.* 2001; **45**(7): 1964-1971
18. Nordmann P, Guibert M. Extended-spectrum β -lactamases in *Pseudomonas aeruginosa*. *J. Antimicrob. Chemother.* 1998; **42**: 128-131